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Chromosome Structural Proteins and RNA-Mediated Epigenetic Silencing

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Structural maintenance of chromosomes (SMC) proteins form the cohesin and condensin complexes and play important roles in sister chromatid pairing, chromosome segregation, and transcriptional regulation. Two papers in *Nature Genetics* now show that SMC-like proteins also participate in epigenetic processes such as X inactivation in mammals and RNA-directed DNA methylation in plants.

Although the stable maintenance of gene silencing through cell division relies on epigenetic marks such as DNA methylation, the actual mechanisms by which such marks are laid down are still poorly understood. Plants use a variety of strategies, including small RNA-directed DNA methylation (RdDM). In mammals, X inactivation represents a classic example of epigenetic silencing, and yet little is known about the manner by which the inactive state is achieved and by which epigenetic changes such as DNA methylation are recruited to promoters of X-linked genes. New findings, based on powerful genetic screens in both plants and mammals, now provide evidence that SMC hinge domain proteins could be important new actors in epigenetic silencing processes.

Blewitt et al. (2008) show that the *s*tructural *m*aintenance of *c*hromosomes *h*inge *d*omain containing 1 (*SmcHD1*) gene plays a critical role in X chromosome inactivation in mice. A mutation in this gene was previously identified as a modifier of transgene silencing in an ENU mutagenesis screen, and found to result in homozygous female-specific lethality at midgestation, indicative of a defect in X inactivation (Blewitt et al., 2005). The authors have now generated SmcHD1 homozygous mutant mice, which they use to explore the role of *SmcHD1* in X inactivation (Figure 1A). In mice, X inactivation occurs in two waves. An initial round of imprinted X inactivation, affecting the paternal X only, begins at the 4- to 8-cell stage. This pattern is maintained in extraembryonic tissues, but reversed in the inner cell mass, where a subsequent wave of random X inactivation takes place in embryonic lineages from around E5.5 (Okamoto and Heard, 2006). Both embryonic and placental defects were observed in *SmcHD1* homozygous mutant female embryos, with death occurring prior to stage E13.5. This is relatively late for an X inactivation phenotype, suggesting that maintenance rather than initiation of X inactivation is compromised. An X-linked GFP transgene showed aberrant transcriptional activity from the inactive X chromosome in both embryonic and extraembryonic cells by E7.5. However, early events in X inactivation, such as Xist RNA coating and Polycomb group protein recruitment, were not affected, suggesting that SmcHD1 acts downstream of these changes during X inactivation. Alternatively, a maternal pool of SmcHD1 protein or mRNA from heterozygous mothers may mask earlier effects in homozygous mutant embryos. To assess the nature of the epigenetic defect on the inactive X chromosome in the *SmcHD1* mutant embryos, the DNA methylation status of multiple X-linked gene promoters was examined. Hypomethylation was found at CpG islands that normally become silenced in response to X inactivation. In some cases partial transcriptional reactivation of the associated gene could be detected. Blewitt et al. therefore propose that *SmcHD1* may specifically affect the efficiency of DNA methylation on the inactive X. However, this cannot explain the X inactivation defects in extraembryonic tissues where X-linked CpG islands tend to remain hypomethylated (Sado et al., 2000). Indeed, the perturbation of DNA methylation at X-linked promoters in tissues of mutant female embryos could simply be a consequence of disrupted transcriptional repression of X-linked genes. Interestingly, in *SmcHD1* heterozygous mice (which are viable), X-linked gene promoter methylation is delayed in onset but reaches normal levels with time, suggesting that SmcHD1 may contribute to the initial deposition of DNA methylation on the inactive X. How, when, and where SmcHD1 is recruited to the inactive X chromosome, and what its exact function might be, are exciting questions for the future.

In a study with tantalizing parallels, this time in plants, Kanno et al. (2008) have discovered that another protein with a SMC hinge-like domain plays a critical role in RdDM. The *dms3* mutant was isolated in an EMS mutagenesis screen of plants containing a viral enhancer-based two-component transgene system. Here, a hairpin RNA expressed from one transgene is processed into siRNAs that target DNA methylation of an enhancer sequence linked to *GFP* on a second transgene. As a result the *GFP* transgene is repressed (Figure 1B). The *dms3* mutant causes a defect in silencing of the transgene. Previous two-component systems, targeting promoter rather than enhancer regions, were used by the same group to isolate a number of mutants in the RdDM pathway (Huettel et al., 2007). The fact that *dms3* was not identified in these genetic screens may provide a clue to its possible function. Indeed, the two-component system used in the new work differs in several ways from previous ones, perhaps most importantly in that the enhancer is transcribed. In previous studies, DNA methylation was restricted to the promoter sequences targeted by the RNA hairpin, while in the

enhancer-based assay, DNA methylation affected not only the target enhancer sequence but also spread downstream into nontargeted regions. Spread of DNA methylation was accompanied by the production of secondary siRNAs from the downstream region, presumably as a result of enhancer transcription. Several models can be envisaged, but it is tempting to speculate that transcription of the enhancer somehow enables recruitment of DMS3, perhaps via siRNAs. Based on the nucleic acid linking ability of SMC proteins, DMS3 might then act as a clamp between nascent RNA and DNA, or nascent RNA and secondary siRNAs, to enable spread of DNA methylation in *cis* beyond the region targeted by the primary siRNAs. The DMS3 protein is much smaller (420 aa) than other functionally characterized SMCs, however, and its function as a "dynamic molecular linker of the genome" (Losada and Hirano, 2005) remains to be established. Whatever its mechanism of action, the authors clearly demonstrate the importance of DMS3 in epigenetic silencing by confirming its role at several natural targets of RdDM, which lose DNA methylation, silencing, or both in the *dms3* mutant. It will be interesting to see whether secondary siRNAs are produced at endogenous targets of DMS3 as in the enhancer-based assay system.

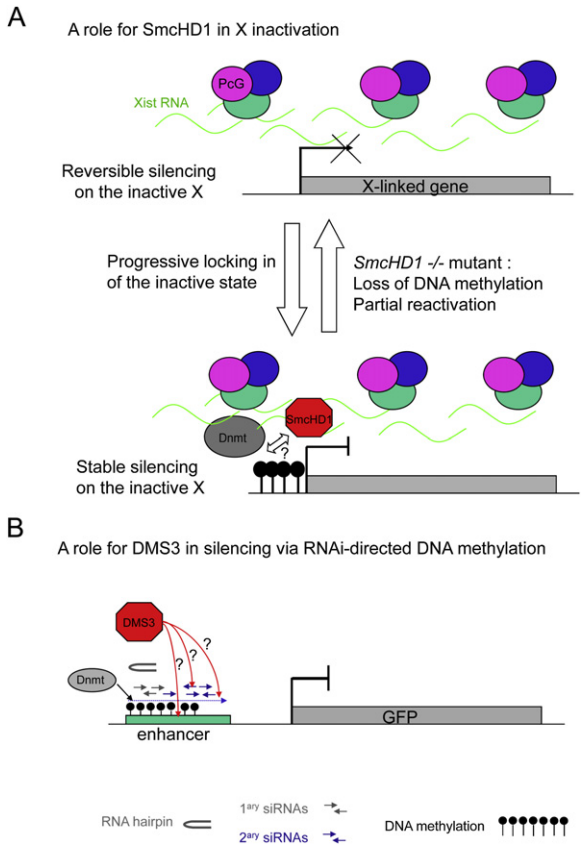

Figure 1. Putative Models of SMC-like Proteins in Epigenetic Processes

(A) Recruitment of SmcHD1 to the inactive X chromosome may involve Xist RNA or chromatin (e.g., Polycomb group proteins at X-linked promoters). As X inactivation shifts from a reversible repressed state to a more stable inactive state, SmcHD1 might participate in this by facilitating or stabilizing recruitment of DNA methyltransferases (DNMtases). Without SmcHD1, loss of DNA methylation and partial reactivation may occur.

(B) A role for DMS3 in RdDM in plants. Expression of an RNA hairpin loop targeting part of an enhancer linked to a GFP reporter gene results in primary siRNA production via RNAi and recruitment of DNMtases (such as DRM2) via RdDM. Transcription of the enhancer, presumably through a cryptic promoter, results in the formation of secondary siRNAs downstream of the primary target sequence. The spread of siRNAs is accompanied by a spread of DNA methylation, which may involve DMS3.

The studies of Blewitt et al. and Kanno et al. add SMC-like proteins to the epigenetic repertoire of higher eukaryotes, linking them to gene silencing processes involving DNA methylation recruitment or spread. SMC proteins were best known for their involvement in chromosome condensation and cohesion, and more recently in gene regulation, particularly gene activation (Peric-Hupkes and van Steensel, 2008). Recent findings, which suggest that cohesins/condensins also participate in heterochromatic maintenance at centomeric repeats in *S. pombe* during G2 as part of a dynamic series of events during the cell cycle, are clearly relevant in this context. SMC proteins could have a critical role following replication in the perpetuation of silent states (Chen et al., 2008; Kloc et al., 2008). Whether the mammalian SmcHD1 and plant DMS3 proteins are recruited to chromatin in a cell-cycle-dependent manner, and whether each proteins' C-terminal hinge domain, which characterizes SMC proteins involved in chromosome condensation and segregation, is actually involved in their epigenetic functions is still not clear. For example the SmcHD1 protein has an N-terminal ATPase domain, which may well participate in its chromatin-associated activity on the inactive X chromosome. Clearly, biochemical analysis of the partners of SmcHD1 and DMS3 and further genetic studies will be crucial for understanding the function of these proteins.

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